



## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<b>(51) International Patent Classification <sup>5</sup> :</b> <b>G01N 33/84, A61K 31/70</b> <b>C12Q 1/02</b>	<b>A1</b>	<b>(11) International Publication Number:</b> <b>WO 94/03815</b> <b>(43) International Publication Date:</b> 17 February 1994 (17.02.94)
<b>(21) International Application Number:</b> PCT/GB93/01621 <b>(22) International Filing Date:</b> 30 July 1993 (30.07.93)  <b>(30) Priority data:</b> 9216360.9                      31 July 1992 (31.07.92)                      GB  <b>(71)(72) Applicant and Inventor:</b> RATCLIFFE, John [GB/GB]; 10 Bolton Grove, Bishop Auckland, County Durham DL14 6LL (GB).  <b>(74) Agent:</b> PERCY, Richard, Keith; Patents Department, British Technology Group Limited, 101 Newington Cause- way, London SE1 6BU (GB).  <b>(81) Designated States:</b> CA, JP, US, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).		<b>Published</b> <i>With international search report.</i>
<b>(54) Title:</b> TREATMENT OF BLOOD SAMPLES  <b>(57) Abstract</b>  Blood samples to be assayed for potassium in serum or plasma are treated soon after the sample has been collected with an energy-providing substance, such as glucose or fructose, which maintains the cellular sodium-potassium pump until the samples are centrifuged. Until then the samples are kept at room temperature.		

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## "TREATMENT OF BLOOD SAMPLES"

### Background of the invention

#### 1. Field of the invention

5        This invention relates to a method of treating a blood sample which has been taken from a human or animal for assay of potassium in plasma or serum.

#### 2. Description of the relevant art

10       Knowledge of the level of potassium in a patient's serum is often useful, for example if the patient is on diuretics or certain heart drugs such as Digoxin.

15       Only a small proportion of the total body potassium is contained in the blood plasma, 98% being located within the cells of the body including the red blood cells. Within the body there is a constant tendency for potassium to diffuse into the extra cellular fluid. This action is opposed by an energy-consuming biochemical process known as the "sodium-potassium pump". Therefore, in the living body, the high concentration of potassium in body cells is maintained.

20       Blood specimens taken from patients within a hospital generally reach the laboratory within one hour but specimens from general practice or peripheral hospitals may not reach the laboratory until the following day.

25       Once the blood sample has been taken, potassium begins to leak out of the blood cells over a period of time because the sodium-potassium pump is not effective. This means that by the time the blood sample is tested, the amount of potassium which was originally in the serum has changed by a large amount. Results of such blood tests therefore are no longer clinically  
30       meaningful.

      Methods have been suggested whereby the problem of significant potassium changes between the compartments of whole blood during delayed transport to the laboratory may be overcome. These include:

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1. Providing each Health Centre/Peripheral Hospital with a centrifuge so that they can separate the serum soon after the blood is taken. Suitable safe centrifuges are very costly. It requires non-laboratory staff to be trained in the technique including safety aspects.
2. Equipping sites and training staff to perform the assay themselves. This would be even more expensive.
3. Opening central laboratories for the handling of specimens during the evening. Again, this is an expensive solution requiring extra transport from all peripheral sites to the central laboratory and increased staffing costs.

It must be emphasised that the present invention is concerned exclusively with the preservation of blood samples from patients, for the purpose of subsequently separating serum or plasma from blood cells and then analysing the serum or plasma for potassium. This is distinct from the preservation of blood samples for haematological purposes, e.g. blood cell counts as in British Specification 1,583,320 (Technicon), analysis of glucose in whole blood as in U.S. Patent 4,833,090 (Liss et al.), the addition of substances to blood intended for transfusion or to the patient's blood in extracorporeal circulation, in either case in order to prevent effects arising from the metabolism of red blood cells such as intravascular haemolysis, post-infusion anaemia and oxyphoretic changes, as in German Offenlegungsschrift 3,323,849 or the preservation of blood cells for transfusion as in the Derwent World Patents Index Abstract Acc. No. 83-727105/31 of Soviet Union Specification No 959,786 (Moscow MD).

#### Summary of the Invention

It has now surprisingly been found that the sodium-potassium pump continues to operate at room temperature provided that an energy-providing substance is added to the blood sample. By such means, the level of potassium in the serum may be maintained at a clinically meaningful level for at least 36 hours provided that

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the blood samples are stored during this period at an appropriate temperature for operation of the sodium-potassium pump, which happens to be approximately room temperature. If, for example, the samples were placed in a refrigerator this would cause the sodium-potassium pump to cease.

The invention is conveniently defined as method of treating a blood sample taken from a patient, which comprises adding to the freshly taken blood sample a substance which provides energy to maintain the sodium-potassium pump, maintaining the sample at a temperature within the range 16 to 30°C, preferably 18 to 25°C, separating plasma or serum from the sample within 72 hours, preferably within 48 hours, of the taking of the blood sample and assaying the plasma or serum for potassium.

#### Brief description of the drawings

Figures 1(a) to (f) are plots of serum potassium levels against time after the taking of blood samples for six different human subjects, for samples in which the blood was untreated and those to which glucose was added to another portion of the same sample;

Figures 2(a) to (f) are similar plots for samples taken from six subjects and divided into three, namely an untreated control and portions treated with glucose and fructose; and

Figures 3 to 5 are similar plots to Figure 2, but each related to a single subject and to three different temperatures of storage of the blood.

#### Description of the preferred embodiments

The energy provider is preferably glucose or fructose, which are very cheap. However, it can be any substance which acts to prevent or mitigate a decrease in the ATP level of the blood sample. Normally, it will be a sugar which is converted in vivo into pyruvate with a net production of 2 moles of ATP per mole of sugar. Pyruvate enters the citric acid (Krebs) cycle and thus provides further energy as ATP. Metabolites of glucose or

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fructose in the glycolic pathway, e.g. the 1-phosphate, 6-phosphate, 1,6-diphosphate, glyceraldehyde-3-phosphate, 1,3-diphosphoglycerate or 3-phosphoglycerate, could be used. Phosphates are preferably in an acidic form.

5 The amount of the energy provider required will obviously depend on the size of the blood sample taken.

The final concentration of the added substance will usually be in the range 12 to 40 millimoles/litre. Lower concentrations than 12 mmol/l. are likely to give diminished effects. A  
10 preferred range is from 12 to 20 millimoles/litre. These concentrations relate only to added substance, i.e. are exclusive of any concentrations already present.

The energy provider may be added to the blood after the sample has been taken, but advantageously it is added  
15 simultaneously to the blood sample by including it in the blood specimen container during manufacture of the container.

The drawings show potassium levels in millimoles/litre on the y-axis plotted against time in hours from the taking of the blood sample on the x-axis. The x-axis is not to scale in order to  
20 avoid excessive elongation of the Figures. Throughout, controls where no addition was made are shown by open circles joined by solid lines, samples treated with glucose by filled squares joined by broken lines and samples treated with fructose by filled circles joined by dotted lines.

25 Referring to Figure 1, blood samples were taken from six subjects. Each of the blood samples was immediately divided into two portions and glucose (to give a final concentration of 3.6mg/ml) of was added to one of these two portions. Each portion was then aliquotted into nine tubes. Each of the  
30 aliquots was kept at room temperature (20°C).

The parallel horizontal chain-link lines on each of the Figures (a) to (f) represents limits within which a variation from the initial result would be clinically insignificant. Measuring the serum concentration of a constituent such as

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potassium in a healthy individual on successive days shows a variation about a mean value. This is chiefly random biological variation and to a lesser extent analytical variation. These variations must therefore be regarded as clinically insignificant. For the average UK laboratory, it has been shown that around the mid-normal points, a difference between the serum potassium in two samples taken at different times of up to 0.6 mmol/litre may have occurred because of biological and analytical variation alone and is therefore clinically insignificant.

10 The nine aliquots of whole blood were centrifuged, at 0.5, 1, 2, 4, 7, 24, 32, 48 and 56 hours after the initial sampling.

Serum was separated from the cells and clotted proteins, by centrifugation, homogenised in a mixer and assayed in the conventional way for potassium, by flame emission photometry.

15 All assays were done in the same batch.

The Figures show that even after a period of 48 hours, the change in the serum potassium in the blood samples to which glucose had been added was clinically insignificant in all six samples. In five of them, it was still insignificant after 56 hours and in the other the potassium level was not seriously elevated. By contrast the serum potassium from untreated blood had changed by an amount which was clinically significant.

20 Referring now to Figure 2, a similar experiment was carried out, again at 20°C, with the variation that the initial sample was divided into three main portions, one control, and two portions to which glucose and fructose, respectively, were added immediately after sampling. Each portion was divided into five aliquots, which were centrifuged at 1, 7, 25, 49 and 73 hours to produce serum. It will be seen that in all six subjects serum potassium levels were scarcely elevated even after 73 hours. Sodium levels were also determined. After 73 hours, they dropped slightly in the controls but remained at approximately the initial value in the treated samples.

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The following Table shows the results of assays of other serum constituents, as analysed on an Ektachem E-700 analyser. The ten samples were those taken from patients for whom various different assays were requested. All the results on the controls  
5 were scaled down by 2% to correct for the volume of the added sugar solution. Results were compared with 2 x the standard errors (S.E.) for the methods which had been calculated previously. As can be seen, there was no statistical evidence for the addition of glucose or fructose to the patients' samples  
10 immediately after taking the samples having any effect on the other assays.



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TABLE: THE EFFECT OF ADDED GLUCOSE AND FRUCTOSE  
ON CLINICAL BIOCHEMISTRY TESTS

5	SAMPLE NUMBER	TEST	CONT.	GLUC.	FRUCT.	S.D.	2 X S.E.
	1	Urea mmol/L	6.7	6.9	6.8	0.2	0.57
		Creatinine $\mu$ mol/L	85	83	84	1.3	3.7
		Sodium mmol/L	135	136	136	1.4	4.0
10		Potassium mmol/L	4.2	4.2	4.2	0.06	0.17
		Chloride mmol/L	97	99	99	1.3	3.7
		CO <sub>2</sub> mmol/L	27	26	26	1.6	4.5
		Amylase IU/L	1380	1348	1351	36	102
		Bilirubin $\mu$ mol/L	25.6	25.3	25.6	1.7	4.8
15		Alk. Phos. IU/L	43	45	45	1.5	4.2
		Gamma-GT IU/L	22.5	22	22	0.5	1.4
		ALT IU/L	23.5	22	21	3.4	9.6
		Total Protein g/L	62.7	62.9	62.5	0.8	2.3
		Albumin g/L	34.3	34.6	34.8	0.5	1.4
20		Calcium mmol/L	2.07	2.10	2.11	0.04	0.11
	2.	CK IU/L	1306	1354	1287	46	130
		AST IU/L	151	153	152	5.3	15
25	3.	Cholesterol mmol/L	7.5	7.7	7.4	0.14	0.4
	4.	Urea mmol/L	19.2	20.0	19.9	0.3	0.85
		Creatinine $\mu$ mol/L	204	214	211	4.0	11
30	5.	CK IU/L	382	383	388	13	37
	6.	Urea mmol/L	38.5	39.8	39.2	0.55	1.5
	7.	Cholesterol mmol/L	7.68	7.99	7.60	0.14	0.4
35		Triglyceride mmol/L	2.29	2.36	2.35	0.04	0.11
	8.	Bilirubin $\mu$ mol/L	36.7	33.5	34.0	1.7	4.8
		Alk. Phos. IU/L	311	341	330	8.4	24
		Gamma-GT IU/L	563	538	545	10.1	29
40		ALT IU/L	58	67	71	8.4	24
		CK IU/L	4.5	397	354	14.2	40
		AST IU/L	257	267	275	9.0	25
	9.	Bilirubin $\mu$ mol/L	41.6	41.1	40.9	1.9	5.4
45	10.	Alk. Phos. IU/L	328	329	321	8.9	25
		Gamma-GT IU/L	550	524	536	10	28
		ALT IU/L	66	69	69	9.6	27
		Protein g/L	48.3	49.0	49.0	0.7	2.0
50		Albumin g/L	27.5	27.5	27.4	0.5	1.4

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## KEY TO TABLE

	Alk. Phos	=	Alkaline phosphatase
	ALT	=	Alanine aminotransferase
5	AST	=	Aspartate aminotransferase
	CK	=	Creatine kinase
	GT	=	Glutamyl transferase
	IU	=	International Units

10 Referring now to Figures 3-5, blood samples were taken from three subjects. Each was divided into three portions, one control, one to which glucose was added and one to which fructose was added, in each case immediately after the sample was taken. Each of the three portions was then aliquotted into

15 six aliquots, which were centrifuged and the serum decanted after 1, 4, 7, 25, 49 and 73 hours. After homogenisation, the aliquots were then assayed all at the same time for serum potassium. The samples from the three subjects were treated differently, being kept at 4°C, 12°C and 30°C respectively. The

20 results shown in Figures 3-5, relating to these respective temperatures, show the elevation of serum potassium in all samples (controls and treated) at 4°C and 12°C, but the beneficial effect of adding glucose or fructose when the blood is kept at 30°C. Based on these results, it is estimated that a

25 suitable temperature at which to keep the blood between taking the sample and separation of the serum is in the range 16-30°C, although the lower end of this range is likely to show some falling away of the benefits of the invention, while 30°C is unnecessarily high. A preferred range is 18-25°C, with 18-22°C

30 being most preferred. Normally assays are done on serum. Since the blood clots serum is obtained simply by separating the liquids from the solids. However, sometimes assays are done on plasma, in which case an anti-coagulant such as heparin is added to the freshly taken samples, whereby the blood-clotting

35 proteins are retained in solution to give plasma which is separated from the cells, conveniently by centrifuging. After the plasma or serum has been separated from the cells, (which is

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normally done by centrifuging, but the method of separation is not critical to the invention) it is permissible to refrigerate the sample if desired.

5 To assay for potassium in serum or plasma, the usual methods are flame emission spectrophotometry or an ion-selective membrane method, as described e.g. by "Textbook of Clinical Chemistry" ed. Norbert W. Tietz., W.B. Saunders (1986), pages 1177-1183. The method used is not critical to this invention.

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## CLAIMS

1. A method of treating a blood sample taken from a patient, which comprises adding to the freshly taken blood sample a substance which provides energy to maintain the sodium-potassium  
5 pump, maintaining the sample at a temperature within the range 16 to 30°C, separating plasma or serum from the sample within 72 hours of the taking of the blood sample and assaying the plasma or serum for potassium.
2. A method according to Claim 1 wherein the energy provider  
10 acts to prevent or mitigate a decrease in the ATP level of the blood sample.
3. A method according to claim 2 wherein the energy provider is glucose, fructose or a metabolite thereof.
4. A method according to claim 3 wherein the metabolite is a 1-  
15 or 6- phosphate or 1, 6-diphosphate.
5. A method according to claim 3 wherein the energy provider is glyceraldehyde-3-phosphate, 1,3-diphosphoglycerate or 3-phosphoglycerate.
6. A method according to any preceding claim wherein the sample  
20 is maintained at 18 to 25°C.
7. A method according to claim 6 wherein the sample is maintained at 18 to 22°C.
8. A method according to any preceding claim wherein the plasma  
25 or serum is separated within 48 hours of the taking of the blood sample.

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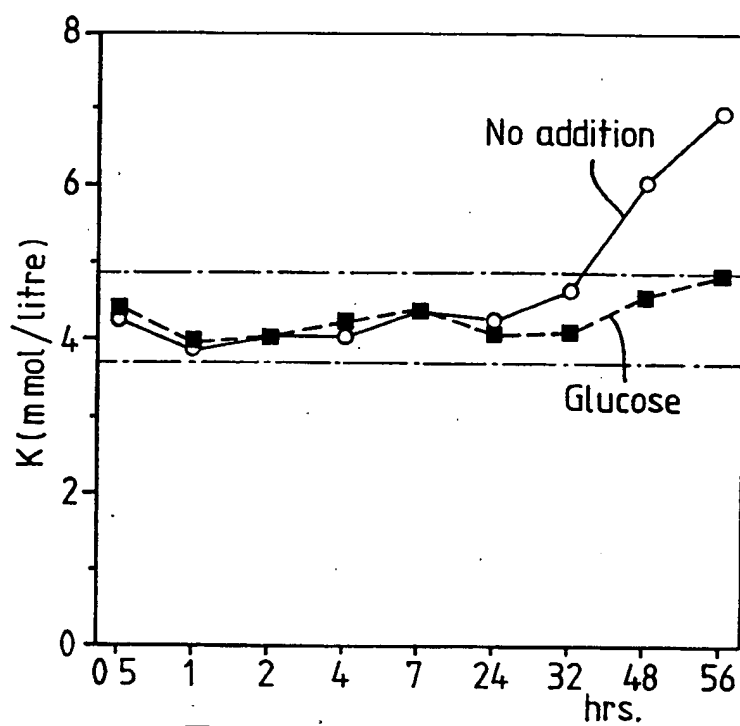


Fig. 1a

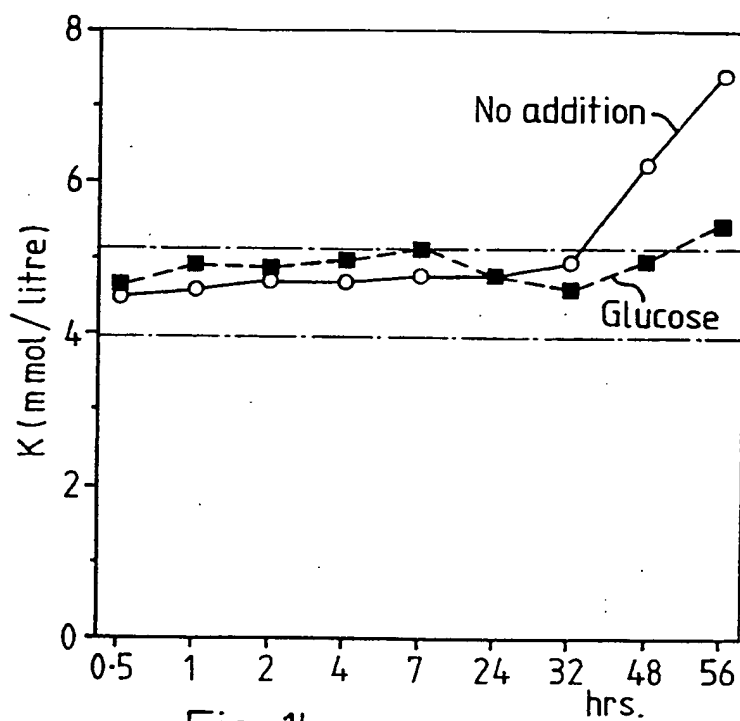


Fig. 1b

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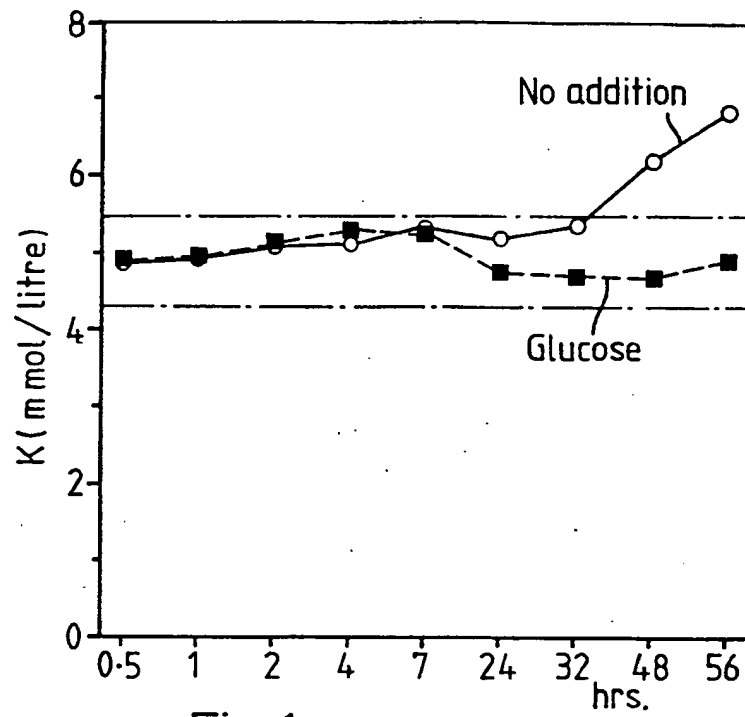


Fig. 1c

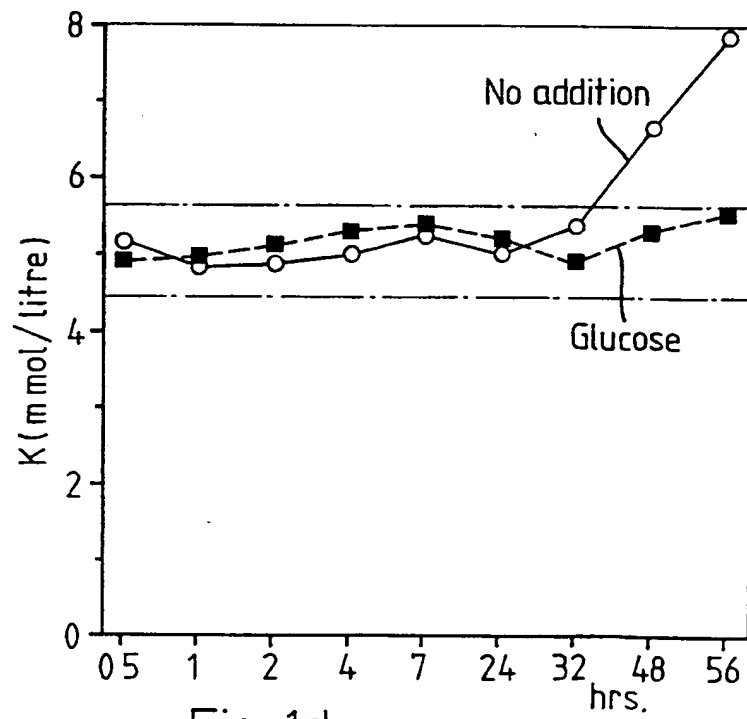


Fig. 1d

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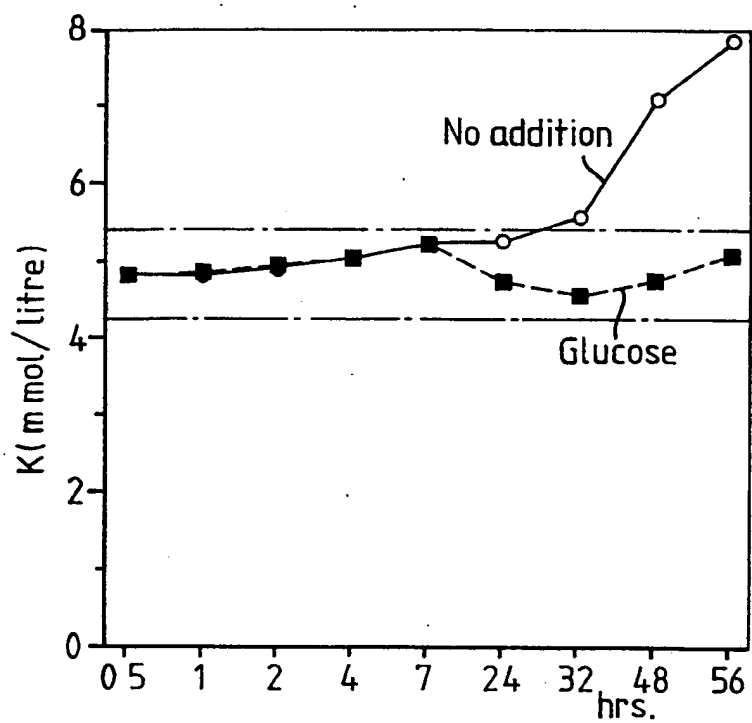


Fig. 1e

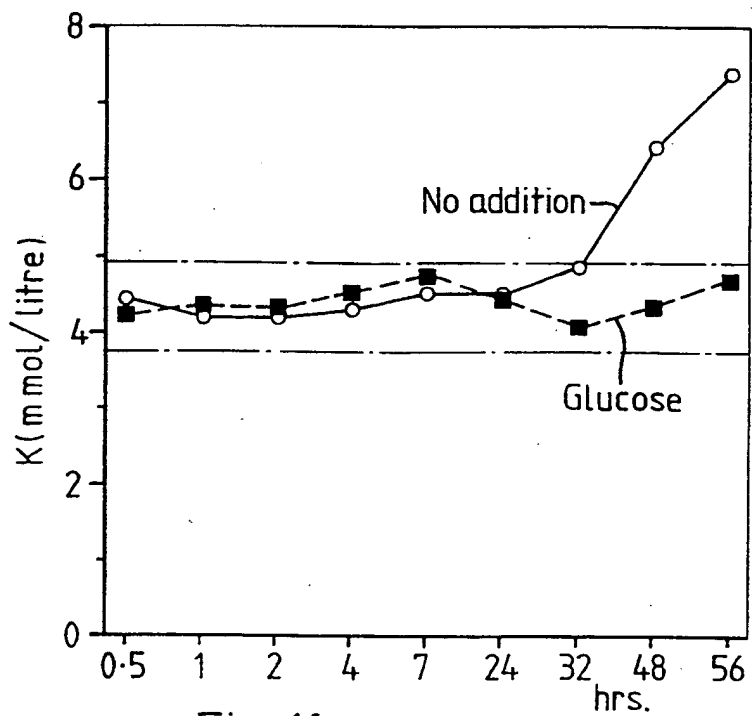


Fig. 1f

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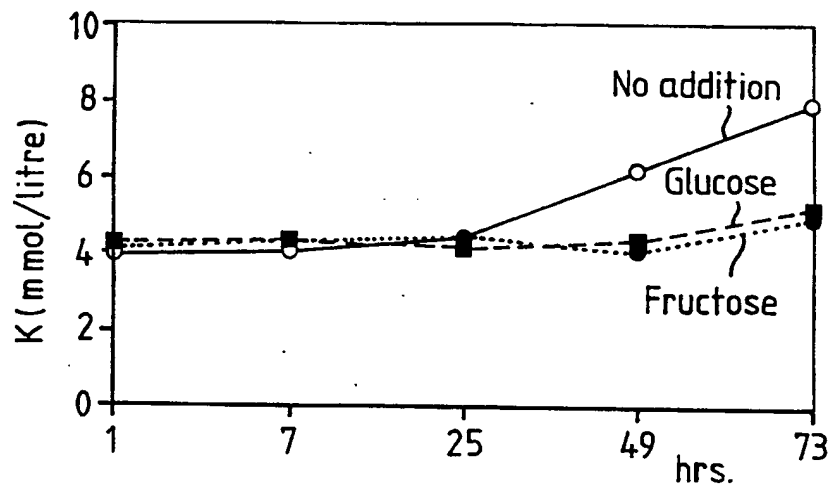


Fig. 2a

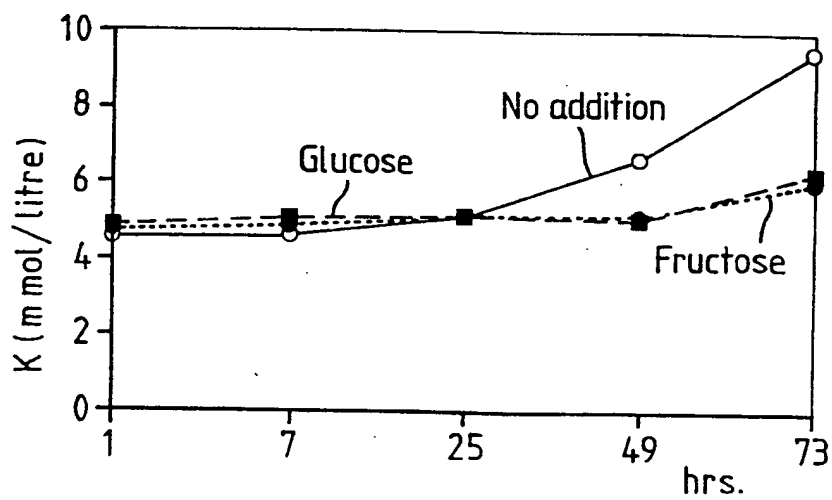


Fig. 2b



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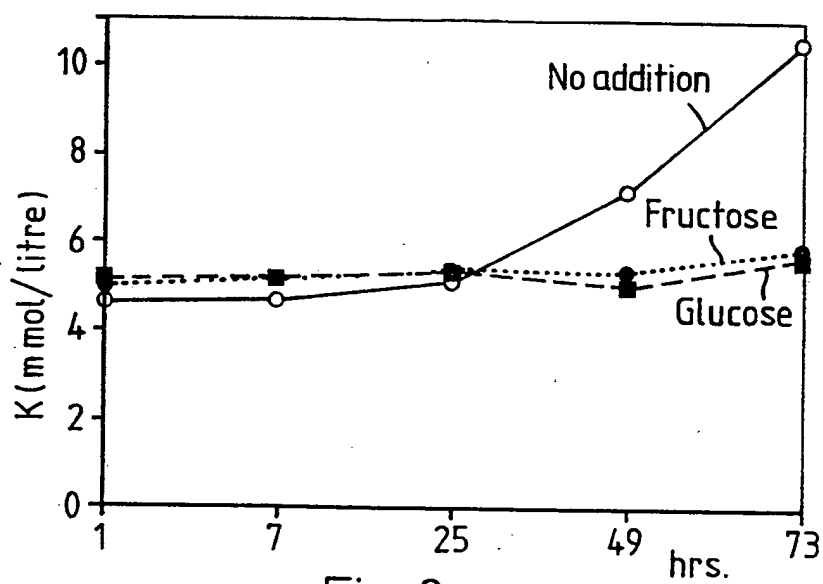


Fig. 2c

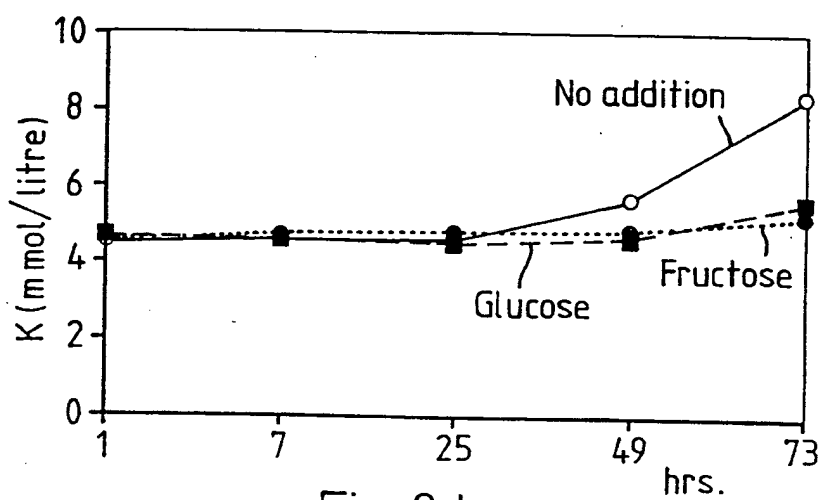


Fig. 2d

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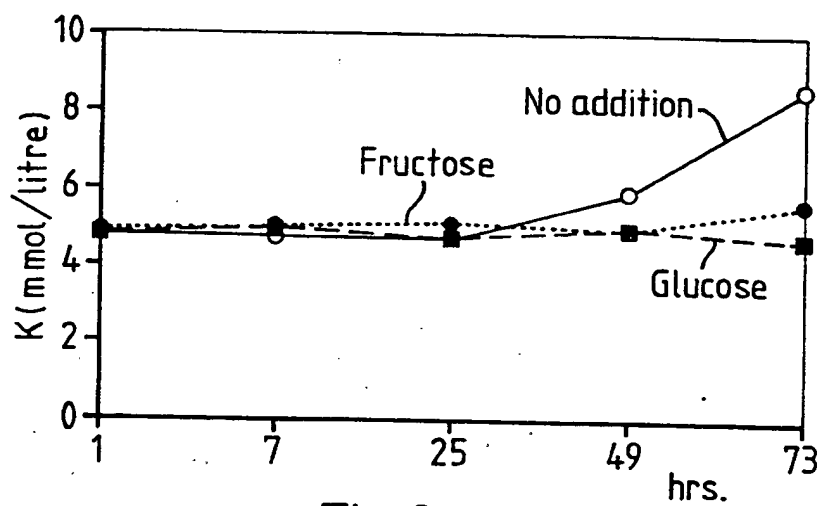


Fig. 2e

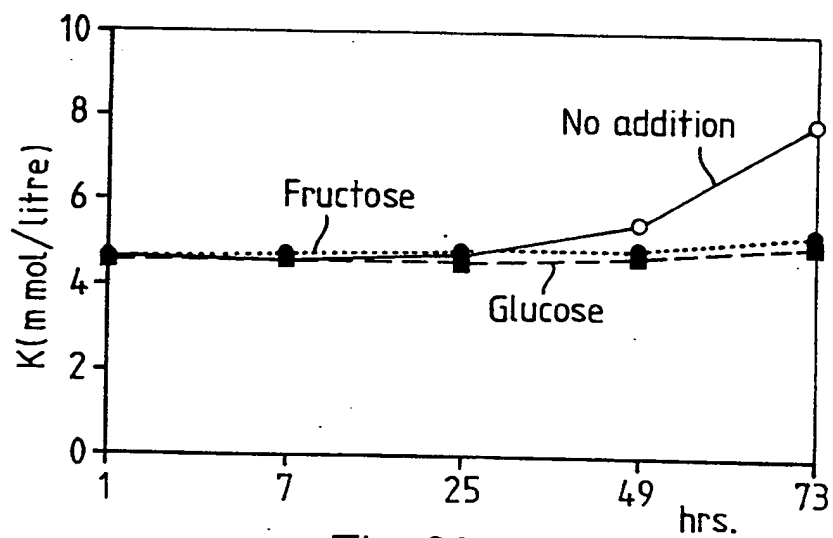


Fig. 2f

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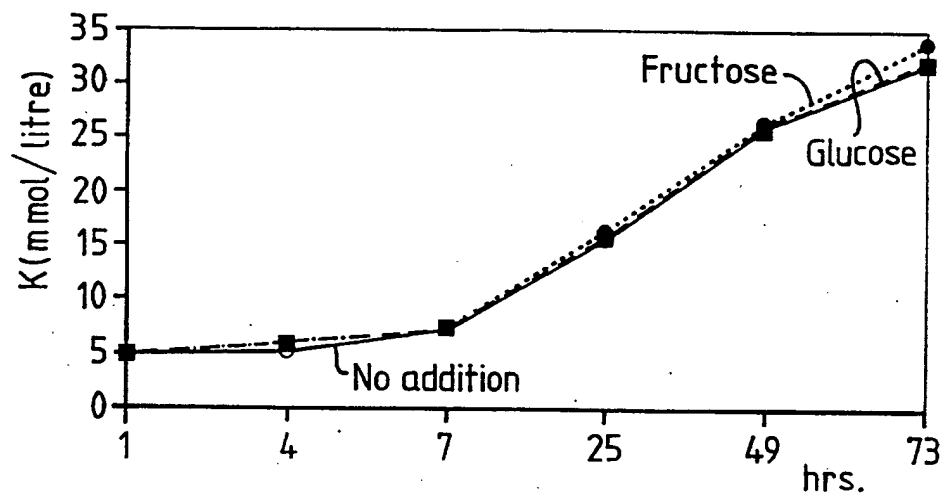


Fig. 3

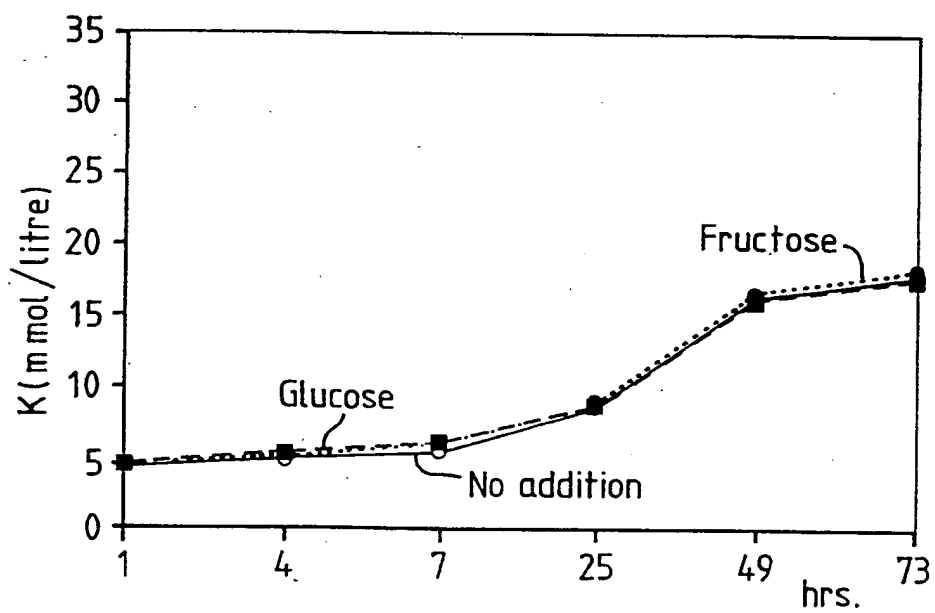


Fig. 4

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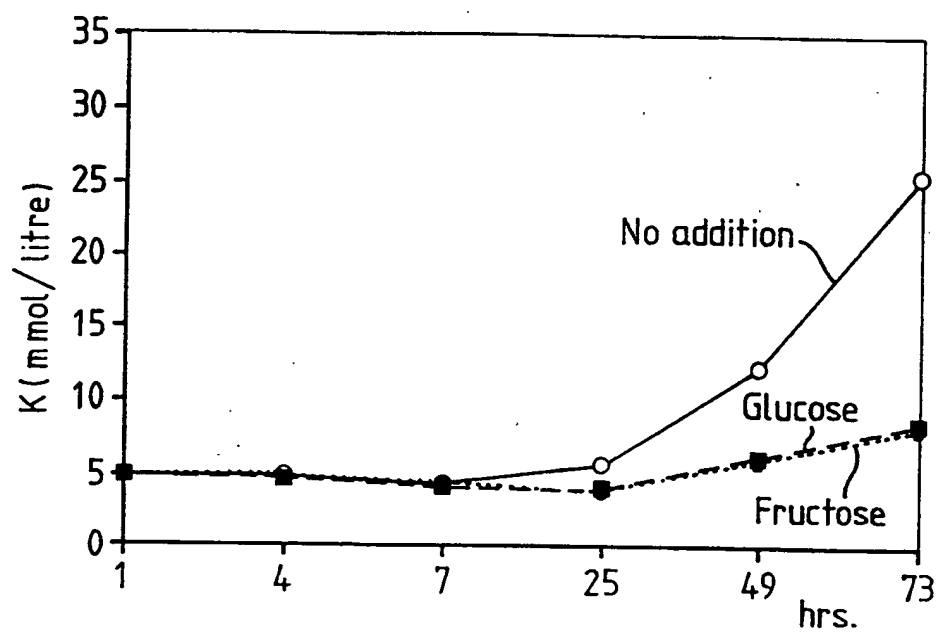


Fig. 5

## INTERNATIONAL SEARCH REPORT

International Application No

PCT/GB 93/01621

<b>I. CLASSIFICATION OF SUBJECT MATTER</b> (If several classification symbols apply, indicate all) *		
According to International Patent Classification (IPC) or to both National Classification and IPC		
IPC <sup>5</sup> : G 01 N 33/84, A 61 K 31/70, C 12 Q 1/02		
<b>II. FIELDS SEARCHED</b>		
Minimum Documentation Searched <sup>7</sup>		
Classification System	Classification Symbols	
IPC <sup>5</sup>	G 01 N 33/00, A 61 K, C 12 Q	
Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched <sup>8</sup>		
<b>III. DOCUMENTS CONSIDERED TO BE RELEVANT</b> <sup>9</sup>		
Category <sup>10</sup>	Citation of Document, <sup>11</sup> with indication, where appropriate, of the relevant passages <sup>12</sup>	Relevant to Claim No. <sup>13</sup>
Y	DE, A1, 3 323 849 (BIOMEDICA FOSCAMA INDUSTRIA CHIMICO-FARMACEUTICA S.P.A.) 03 January 1985 (03.01.85), abstract, claims 1,2.	1,4
Y	EP, A2, 0 141 134 (ABBOTT LABORATORIES) 15 May 1985 (15.05.85), abstract; page 1, lines 12-29.	1,4
P, A	CHEMICAL ABSTRACTS, vol. 117, no. 19, issued 1992, November 09 (09.11.92) (Columbus, Ohio, USA), M. MARJANOVIC et al. "ATP dependence of sodium-potas- sium pump of cold-sensitive and cold-tolerant mammalian red blood cells", page 516, abstract no. 188988q	1,2
<p>* Special categories of cited documents: <sup>14</sup></p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> <p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step</p> <p>"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art</p> <p>"Z" document member of the same patent family</p>		
<b>IV. CERTIFICATION</b>		
Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report	
12 October 1993	12. 11. 93	
International Searching Authority	Signature of Authorized Officer	
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III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)		
Category *	Citation of Document, " with indication, where appropriate, of the relevant passages	Relevant to Claim No.
A	<p>&amp; J. Physiol. (London) 1992, 456, 575-90.</p> <p>--</p> <p>CHEMICAL ABSTRACTS, vol. 96, no. 3, issued 1982, January 18 (Columbus, Ohio, USA), R.W. MERCER et al. "Membrane-bound ATP fuels the sodium/potassium pump. Studies on membrane-bound glycolytic enzymes on inside-out vesicles from human red cell membranes", page 265, abstract no. 17853k &amp; J. Gen. Physiol. 1981, 78(5), 547-68.</p> <p>--</p>	1,2
A	<p>CHEMICAL ABSTRACTS, vol. 95, no. 23, issued 1981, December 07 (Columbus, Ohio, USA), R.W. MERCER et al. "Biphasic effect of orthophosphate on the sodium-potassium pump of human red cells", page 448, abstract no. 201330t &amp; Biochim. Biophys. Acta 1981, 648(1), 63-70.</p> <p>--</p>	1
A	<p>CHEMICAL ABSTRACTS, vol. 111, no. 19, issued 1989, November 06 (Columbus, Ohio, USA) M.A. YOREK et al. "Effect of galactose and glucose levels and sorbinil treatment on myo-inositol metabolism and sodium-potassium pump activity in cultured neuroblastoma cells", page 516, abstract no. 171883e &amp; Diabetes 1989, 38(8), 996-1004.</p> <p>--</p>	1,3
A	<p>CHEMICAL ABSTRACTS, vol. 109, no. 11, issued 1988, September 12 (Columbus, Ohio, USA), M.A. YOREK et al. "Effect of</p>	1,3

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)		
Category *	Citation of Document, " with indication, where appropriate, of the relevant passages	Relevant to Claim No.
	<p>increased glucose levels on sodium/potassium pump activity in cultured neuroblastoma cells", page 508, abstract no. 90708h &amp; J. Neurochem. 1988, 51(2), 605-10.</p> <p>-----</p>	

## ANHANG

## ANNEX

## ANNEXE

zum internationalen Recherchen-  
bericht über die internationale  
Patentanmeldung Nr.

to the International Search  
Report to the International Patent  
Application No.

au rapport de recherche inter-  
national relatif à la demande de brevet  
international n°

PCT/GB 93/01621 SAE 77557

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Im Recherchenbericht angeführtes Patentdokument Patent document cited in search report Document de brevet cité dans le rapport de recherche	Datum der Veröffentlichung Publication date Date de publication	Mitglied(er) der Patentfamilie Patent family member(s) Membre(s) de la famille de brevets	Datum der Veröffentlichung Publication date Date de publication
DE A1 3323849	03-01-85	keine - none - rien	
EP A2 141134	15-05-85	US A 4639424 AT E 38098 AU A1 33221/84 AU B2 567185 CA A1 1219795 DE C0 3474724 DK A0 4497/84 DK A 4497/84 EP A3 141134 EP B1 141134 ES A1 536948 ES A5 536948 ES A1 8605642 GR A 80701 IL A0 72721 IL A1 72721 JP A2 60102561 NZ A 209349 ZA A 8406513	27-01-87 15-11-88 26-04-85 12-11-87 31-03-87 24-11-88 20-09-84 22-04-85 10-07-85 19-10-88 16-03-86 16-04-86 01-09-86 18-02-85 30-11-84 30-10-87 06-06-85 06-03-87 24-04-85